

The primary mesenchyme cells (PMCs) form the skeleton in sea urchin larvae and provide a good model system for studying specification, cell signaling, and morphogenesis. A PMC gene regulatory network (GRN) has been built experimentally and shows the relationship between transcription factors (TFs) that direct PMC specification and skeletogenesis. Although advanced, some connections of the PMC GRN require further study. FoxN2/3, a Forkhead TF, is known to be expressed in the PMCs, but its function and regulation is not well known. Here we show FoxN2/3 plays an important role in PMC specification and ingression. Knockdown of FoxN2/3 using an antisense morpholino initially inhibits PMC ingression. Although these embryos eventually gastrulate, the resulting larval skeletons become truncated and disorganized. FoxN2/3 regulates several PMC specific genes involved in the biomineralization including Sm30 and Sm50. FoxN2/3 expression begins at the early blastula stage in the vegetal plate (the territory of the PMCs) but is no longer detectable after ingression. FoxN2/3 expression shifts in the vegetal plate to the non-skeletogenic mesoderm and later to the endoderm. This moving torus pattern of gene expression is similar to that of Blimp1b whose regulatory mechanism is well known. However, the knockdown either of Blimp1b or FoxN2/3 suggests that they have independent regulatory subcircuits. Perturbation experiments show FoxN2/3 is regulated by Pmar1, one of the most upstream TFs of the PMC GRN, and Tbr, a T-box TF. In summary, our findings suggest that FoxN2/3 plays a crucial role in PMC specification and ingression and is regulated by early acting PMC specification genes.

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Program/Abstract # 146

Sea urchin embryonic skeletogenesis is regulated by microRNA

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The Gene Regulatory Network (GRN) driving the first 30 h of embryonic development in the sea urchin is at an advanced state of understanding. Most of the transcription factors and signaling mechanisms active in the embryo are included in the current endomesoderm and ectoderm GRN models. As yet, however, there has been little work done on the role of post-transcriptional regulation in determining GRN behavior. Of particular interest are the roles miRNA may play in modulating the function of the GRN. miRNAs are short (~21–23nt) noncoding RNAs that negatively regulate the translation of target mRNAs by binding to their 3' UTR. Recent studies demonstrated that miRNA function in later developmental events, but there are few studies examining miRNA function in early embryonic processes. All the genes necessary for miRNA synthesis and function are expressed in the sea urchin embryo, and the sea urchin genome contains several hundred predicted miRNA sequences. To begin to understand the role of miRNA in sea urchin embryogenesis, we characterized embryos in which Dicer (a protein required for miRNA synthesis) expression was knocked down using morpholino antisense oligonucleotides. In these embryos, development is delayed, and formation of the embryonic skeleton is completely blocked, demonstrating a requirement for post-transcriptional regulation in the skeletogenic lineage. We are currently characterizing the molecular basis of this phenotype, using the known transcription factors and signaling mechanisms in the skeletogenic GRN as a guide in order to determine exactly how miRNA regulation affects development in the early embryo.

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Program/Abstract # 147

Developmental timing genes identified through miRNA suppressor screens in *C. elegans*

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In the formation of an organism from a single cell, developmental events must be temporally regulated to ensure proper patterning of the adult. In *Caenorhabditis elegans*, timing is regulated by the heterochronic genes, and disruption of these genes leads to skipping or reiteration of certain developmental events. Among these genes are the first-described miRNAs, lin-4 and let-7. Loss of function for either of these genes causes a “retarded” phenotype in which developmental events are reiterated in subsequent stages, delaying differentiation of adult tissues. Several miRNAs, including miR-48, miR-241, and miR-84, share identity with the 5' end of let-7 miRNA, and may target an overlapping set of mRNAs. Disruption of these three let-7 sisters together results in a retarded phenotype. Gain-of-function alleles of mir-48 were recovered as suppressors of lin-4 retarded phenotypes, and cause a precocious phenotype. Overexpression of mir-48 from multicopy arrays leads to enhanced precocious defects, including aberrations in vulva precursor cell divisions, resulting in disruption of egg-laying. To identify additional players in the pathway, we screened for suppressors of mir-48 overexpression that restored egg-laying. These screens are expected to identify new heterochronic mutants, miR-48 target genes, and genes involved in miRNA expression and function. We isolated 36 suppressed lines from 48,000 haploid genomes screened. Preliminary analysis indicates some of the suppressor alleles on their own result in retarded phenotypes, validating this approach for identification of new regulators of developmental timing.

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Program/Abstract # 148

The effects of resveratrol on lifespan extension and gene expression in *Drosophila melanogaster*

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Caloric restriction extends lifespan in a variety of organisms including mice, worms, yeast and flies. The polyphenol resveratrol can be found in the skin of grapes and several studies have shown that dietary exposure mimics caloric restriction. Resveratrol may extend life span by activating the NAD⁺ dependent deacetylase sirtuins although its role on gene expression remains unclear. To replicate published studies and further understand the effect of resveratrol on gene expression during lifespan extension, we are exposing *Drosophila melanogaster* to 0 μ M, 100 μ M and 350 μ M concentrations of resveratrol in food. Survival curves are being generated for isogenized adult males and microarray analysis will be conducted to identify genes that are differentially expressed under the various concentrations of resveratrol.

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Program/Abstract # 149

Downstream targets of Atoh1 (Math1)

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